Journal of Clinical Lipidology (2015) 9, 247–255

Journal of Clinical Lipidology

# Discordance between apolipoprotein B and low-density lipoprotein particle number is associated with insulin resistance in clinical practice

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**BACKGROUND:** Discordance between measures of atherogenic lipoprotein particle number (apolipoprotein B [ApoB] and low-density lipoprotein [LDL] particle number by nuclear magnetic resonance spectroscopy [LDL-$P_{NMR}$]) is not well understood. Appropriate treatment considerations in such cases are unclear.

**OBJECTIVES:** To assess discordance between apoB determined by immunoassay and LDL-$P_{NMR}$ in routine clinical practice, and to characterize biomarker profiles and other clinical characteristics of patients identified as discordant.

**METHODS:** Two retrospective cohorts were evaluated. First, 412,013 patients with laboratory testing performed by Health Diagnostic Laboratory, Inc., as part of routine care; and second, 1411 consecutive patients presenting for risk assessment/reduction at 6 US outpatient clinics. Discordance was quantified as a percentile difference (LDL-$P_{NMR}$ percentile − apoB percentile) and attainment of percentile cutpoints (LDL-$P_{NMR}$ ≥ 1073 nmol/L or apoB ≥ 69 mg/dL). A wide range of cardiovascular risk factors were compared.

**RESULTS:** ApoB and LDL-$P_{NMR}$ values were highly correlated ($R^2$ = 0.79), although substantial discordance was observed. Similar numbers of patients were identified as at-risk by LDL-$P_{NMR}$ when apoB levels were < 69 mg/dL (5%-6%) and by apoB values when LDL-$P_{NMR}$ was < 1073 nmol/L (6%-7%). Discordance (LDL-$P_{NMR}$ > apoB) was associated with insulin resistance, smaller LDL particle size, increased systemic inflammation, and low circulating levels of "traditional" lipids, whereas discordance (apoB > LDL-$P_{NMR}$) was associated with larger LDL particle size, and elevated levels of lipoprotein(a) and lipoprotein-associated phospholipase A2 (Lp-$PLA_2$).

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Submitted July 11, 2014. Accepted for publication November 23, 2014.



http://dx.doi.org/10.1016/j.jacl.2014.11.005

**CONCLUSION:** Discordance between apoB and LDL-$P_{NMR}$ in routine clinical practice is more widespread than currently recognized and may be associated with insulin resistance.


## Introduction

Calculated or measured low-density lipoprotein cholesterol (LDL-C) concentration has long been the major goal of lipid-modulating therapies.[1] However, many individuals experience cardiovascular disease (CVD)-related events or progression of atherosclerosis despite having optimal LDL-C; in fact, half of all patients hospitalized with coronary artery disease have LDL-C levels at or below previously recommended targets.[2] This residual or "hidden" risk—not identifiable by measuring LDL-C—contributes substantially to CVD-related morbidity and mortality and underscores the need for identification of prognostic lipoprotein parameters that transcend cholesterol content. Recent recommendations promulgated in the American College of Cardiology/American Heart Association Treatment of Blood Cholesterol guidelines noted the lack of high-level evidence supporting continued treatment to specific LDL-C target values.[3] In 2006, a group of 30 experts from 10 different countries published a consensus paper[4] citing multiple large studies, including the Apoprotein-related Mortality Risk Study[5] and the INTERHEART Study,[6] noting that the potential for future CVD events is better reflected by measures of circulating atherogenic particle concentration—specifically, apoB—than by LDL-C.

ApoB, of which there is one copy per lipoprotein particle, is found on several potentially atherogenic lipoproteins, including LDL, very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), and lipoprotein(a) (Lp(a)); however, because of the long plasma residence time of LDL compared with that of VLDL and IDL, 90% or more of all apoB is associated with LDL. ApoB measurement therefore provides a reasonable approximation of circulating LDL particle concentration (LDL-P).[7] Most apoB assay methods are immunoassays in which specific antibodies are used to precipitate apoB in the liquid phase, with the immunocomplexes then quantitated using turbidimetric or nephelometric approaches. Among methods available for LDL-P measurement, that using nuclear magnetic resonance spectroscopy (LDL-$P_{NMR}$) is by far the best validated, and the only assay cited by organizational position statements. On the basis of clinical trial data, these statements have advocated the use of either (or both) apoB and LDL-$P_{NMR}$.[8–14] The latter biomarker is supported by multiple large prospective cohort studies that have monitored clinical outcomes, including the Women's Health Study,[15–17] the Framingham Offspring Heart Study,[18] and the Multi-Ethnic Study of Atherosclerosis.[19] LDL-P also appears to be the better indicator of subclinical CVD because it associates more strongly with coronary calcium or carotid intima-media thickness than does LDL-C.[20,21]

The superiority of apoB and LDL-P for cardiovascular (CV) risk assessment is most evident when LDL-C and LDL-P/apoB are discordant—a phenomenon that is especially prevalent among individuals with components of the metabolic syndrome or evidence of insulin resistance[22,23] and in those taking medications, such as statins, that reduce LDL-C to a greater extent than LDL-P.[10,24] In such settings, LDL-C, compared with apoB or LDL-P, has been shown to significantly mischaracterize CVD risk.[25,26] Because those with discordantly high LDL-P or apoB tend to have small, cholesterol-depleted LDL and other evidence of insulin resistance, consideration of particle number helps identify high-risk individuals who would have otherwise been overlooked because of their "optimal" LDL-C levels, an estimated 34% of the population.[27]

Importantly, the relationship between LDL-P or apoB and CV events is maintained on treatment. Although statins can effectively lower LDL-C in individuals with a wide spectrum of CV risk, they reduce CV event incidence by only 25%-30%.[28] This substantial residual risk may be partly attributed to the fact that statin treatment unequally affects LDL-C and LDL-P or apoB levels, reducing LDL-C to the 21st percentile of the population but reducing apoB and LDL-$P_{NMR}$ to only the 55th and 51st percentiles, respectively.[26] Accordingly, many clinical trials of lipid-lowering agents have demonstrated that future CV events are strongly predicted by on-treatment apoB and LDL-$P_{NMR}$, but not LDL-C; apoB and LDL-$P_{NMR}$ thus provide a more reliable assessment of treatment efficacy.[29–33] The use of LDL-$P_{NMR}$ to guide patient care has been shown to result in lower health care costs, fewer CV events, and greater improvements in lipoprotein profile than the use of LDL-C alone.[34]

Although most studies evaluating both apoB and LDL-$P_{NMR}$ have demonstrated that these measures are similarly associated with clinical outcomes, a recent review of 25 studies including 85 clinical outcomes concluded that outcomes were significantly associated with both biomarkers in 58.8% of comparisons, with neither in 20%, and with only 1 biomarker in 21.1%.[35] In a more in-depth critique of this review, Master and Rader suggest that although apoB and LDL-$P_{NMR}$ were comparable at the level of bare statistical association with CV events, LDL-$P_{NMR}$ was more strongly related to outcomes than was apoB in the majority of studies.[36] The present study was conducted to determine the extent of discordance between apoB and LDL-$P_{NMR}$ in a large patient cohort, to characterize the biomarker profiles of patients with discordant apoB and LDL-$P_{NMR}$, and to evaluate the potential role of treatment

effects and insulin resistance in a subset of patients for whom additional data were available.

## Methods

### Subjects

Discordance between LDL-$P_{NMR}$ and apoB was investigated in 2 different retrospective cohorts. The first consisted of deidentified laboratory data from 412,013 consecutive, unique patients who had laboratory testing performed by Health Diagnostic Laboratory (HDL, Inc.) as part of routine clinical care. Only laboratory data, age, and gender were available for these patients. The second cohort was from a retrospective study of 1411 consecutive patients presenting for risk assessment and risk reduction at 6 outpatient clinics across the United States. Family and medical history, current medications, vital signs, and demographic information were collected from chart review and matched to laboratory biomarker data, which was then deidentified. The study protocol was approved and waivers of informed consent were granted by the Copernicus Group Institutional Review Board (Durham, NC).

### Laboratory measurements

Blood samples were drawn after an overnight fast and shipped with cold packs to HDL, Inc. for biomarker testing. Samples were prepared at each clinical site according to standardized instructions appropriate for specimen type (BD Vacutainer SST "Tiger Top" tubes were used for all tests except Vacuette Z Serum Sep. Clot Activator "Bumble Bee Top" tubes used for NMR analysis and BD Vacutainer K2 Whole Blood "Lavender Top" tubes used for HbA1c), received at HDL, Inc. within 24 hours, and tested immediately. ApoB was analyzed using an immunoturbidimetric assay from Roche Diagnostics (Indianapolis, IN) on a Roche/Hitachi P-Modular system. LDL-C and high-density lipoprotein cholesterol (HDL-C) were measured using direct enzymatic assays (Beckman-Coulter Biomedical Ltd., Co. Clare, Ireland). LDL-$P_{NMR}$, high-density lipoprotein particle number (HDL-P), and other lipoprotein parameters were measured at LipoScience (Raleigh, NC) as described previously.[16] Triglyceride assay was performed using standard automated enzymatic methods (Roche Diagnostics), high-sensitivity C-reactive protein (hs-CRP) by immunoturbidimetric assay (Roche Diagnostics), and lipoprotein-associated phospholipase A2 (Lp-$PLA_2$) by enzyme-linked immunosorbent assay (PLAC Test ELISA kit; diaDexus, Inc., San Francisco, CA). Metabolic biomarkers were measured as follows: fasting glucose by an ultraviolet method (Roche Diagnostics) on a Beckman AU5800 analyzer; HbA1c by high-performance liquid chromatography (VARIANT II TURBO $HbA_{1c}$ Kit; Bio-Rad, Hercules, CA); leptin and proinsulin by enzyme-linked immunosorbent assay (Mercodia, Inc., Winston-Salem, NC) on a DSX analyzer; adiponectin by latex turbidimetric immunoassay (MedtestDx, Canton, MI) on a Beckman AU5800 analyzer; free fatty acids by an enzymatic colorimetric method (Wako Chemicals USA, Inc., Richmond, VA) on a Beckman AU5800 analyzer; ferritin by a sandwich principle method (latex agglutination; Roche Diagnostics); and insulin and C-peptide by electrochemiluminescence immunoassay (Roche Diagnostics) on a Roche E module analyzer. The leptin-to-adiponectin (leptin:adiponectin) ratio was calculated as leptin (ng/mL)/adiponectin (μg/mL). The homeostatic model assessment of insulin resistance (HOMA-IR), a surrogate measure of insulin resistance, was calculated as: glucose (mg/dL) × insulin/405 (μU/mL).

### Statistical analysis

Two methods were used to characterize discordance. First, an ApoB/LDL-$P_{NMR}$ discordance score (DS) was calculated for each patient. LDL-$P_{NMR}$ and apoB values were converted into percentiles and subtracted (DS = apoB percentile − LDL-$P_{NMR}$ percentile), providing a normalized measure of discordance. Differences between DS quintiles were assessed with one-way analysis of variance (ANOVA) or Pearson chi-square tests, and Spearman rank correlation coefficients were calculated to evaluate the linear relationship between DS and each dependent measure. All dependent measures with non-normal distributions were log-transformed. Significant ANOVA results were followed with Bonferroni-adjusted Dunnett tests comparing the first and fifth quintiles (most discordant) with the third (most concordant).

Second, discordance was defined as agreement (Y/N) with reference to population cutpoints established within the large cohort. Specifically, the 20th percentile of apoB (69 mg/dL) and LDL-$P_{NMR}$ (1073 nmol/L) were used in this analysis to define potential risk thresholds. All subjects were then

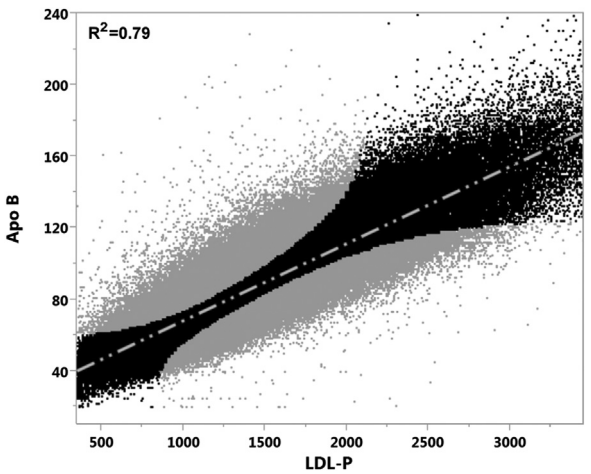


**Figure 1** Relationship between low-density lipoprotein particle concentration (LDL-P; nmol/L) and apolipoprotein B (apoB; mg/dL) values in 412,013 consecutive patients. Lightly shaded areas indicate the first (discordant LDL-P > apoB, bottom right) and fifth (discordant apoB > LDL-P, top left) quintiles of discordance scores.

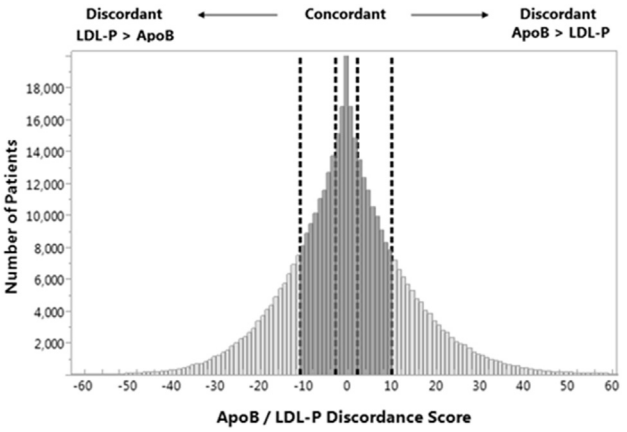


**Figure 2** Distribution of the apolipoprotein B (apoB)/low-density lipoprotein particle concentration (LDL-P) discordance score (apoB percentile − LDL-P percentile), N = 412,013. Dashed lines indicate quintile boundaries.

classified in one of four categories: both low, discordant (LDL-$P_{NMR}$ > apoB), discordant (apoB > LDL-$P_{NMR}$), or both high. Differences in biomarkers and other parameters between clinical risk categories were assessed with 1-way ANOVA or Pearson chi-square tests as appropriate. Significant results were followed with Bonferroni-adjusted Dunnett tests comparing groups against the "both low" group. The smaller cohort was evaluated in the same manner. Patients currently taking exogenous insulin (n = 189) were excluded from analysis of insulin levels (and HOMA-IR) to avoid confounding effects.

## Results and discussion

### Characterization of ApoB/LDL-$P_{NMR}$ discordance in a large clinical dataset

As expected, LDL-$P_{NMR}$ and apoB were strongly correlated ($R^2$ = 0.79; Fig. 1). The median values for LDL-$P_{NMR}$ and apoB in this large cohort were 1459 nmol/L and 88 mg/dL, respectively—comparable to the 50th percentiles reported in the Framingham Offspring Study.[2] Despite this strong correlation, a high degree of discordance was observed. As shown in Figure 2, the discordance scores (apoB percentile − LDL-$P_{NMR}$ percentile) were normally distributed (skewness = 0.2, kurtosis = 1.4), with the first and fifth quintiles trailing off either side of the mean and showing at least a 10-percentile-unit difference. The light shading in Figure 1 illustrates the actual values of LDL-$P_{NMR}$ and apoB for the first quintile (discordant

**Table 1** Patient characteristics (±SD) by quintiles of the ApoB/LDL-P discordance score (apoB percentile − LDL-P percentile), N = 412,013

| | ApoB/LDL-P discordance score quintiles | | | | | |
|---|---|---|---|---|---|---|
| Patient characteristics | First (LDL-P > ApoB) | Second | Third | Fourth | Fifth (ApoB > LDL-P) | Linear trend (Spearman's rho) |
| Demographics | | | | | | |
| Gender (% F) | 47.8* | 47.9 | 49.3 | 50.8 | 55.9* | — |
| Age | 58.3 (13.9)* | 57.3 (14.3) | 56.7 (14.7) | 56.6 (14.9) | 56.8 (14.9)* | −0.04† |
| Lipids | | | | | | |
| TCHOL (mg/dL) | 161 (29)* | 175 (39) | 194 (57) | 190 (41) | 195 (36)* | 0.30† |
| LDL-C (mg/dL) | 86.1 (20.5)* | 96.2 (29.8) | 109.4 (45.5) | 106.2 (34.6) | 105.9 (26.9)* | 0.23† |
| HDL-C (mg/dL) | 46.8 (11.9)* | 49.3 (12.6) | 52.2 (13.7) | 55.0 (15.2) | 60.4 (18.2)* | 0.31† |
| TG (mg/dL) | 132 (75)* | 139 (8) | 152 (101) | 138 (87) | 143 (146)* | −0.02† |
| Lipoproteins/apolipoproteins | | | | | | |
| ApoB (mg/dL) | 80.1 (15.6)* | 88.0 (24.0) | 99.1 (37.9) | 94.7 (26.4) | 94.9 (18.1)* | 0.22† |
| LDL-P (nmol/L) | 1596 (366)* | 1603 (555) | 1693 (778) | 1456 (484) | 1276 (309)* | −0.24† |
| LDL size (nm) | 20.5 (0.5)* | 20.6 (0.5) | 20.7 (0.6) | 20.9 (0.6) | 21.0 (0.6)* | 0.32† |
| ApoA-I (mg/dL) | 140 (28)* | 142 (28) | 146 (29) | 150 (30) | 158 (33)* | 0.21† |
| HDL-P (μmol/L) | 33.7 (7.4)* | 34.0 (7.1) | 34.0 (7.1) | 34.9 (7.1) | 36.0 (7.3)* | 0.11† |
| HDL size (nm) | 8.6 (0.4)* | 8.7 (0.4) | 8.8 (0.5) | 8.9 (0.5) | 9.0 (0.6)* | 0.29† |
| Lp(a) mass (mg/dL) | 31.4 (37.5)* | 32.3 (38.2) | 33.7 (39.7) | 35.1 (40.6) | 37.2 (42.9)* | 0.05† |
| Inflammation/metabolic | | | | | | |
| hs-CRP (mg/L) | 4.8 (10.2)* | 4.3 (8.1) | 4.2 (8.2) | 3.7 (6.94) | 3.6 (8.2)* | −0.08† |
| Lp-$PLA_2$ (ng/mL) | 136 (41)* | 142 (41) | 147 (42) | 154 (42) | 162 (43)* | 0.23† |
| Insulin (μU/mL) | 17.0 (21.3)* | 16.2 (19.8) | 15.5 (19.2) | 14.5 (17.7) | 13 (16.8)* | −0.14† |

ApoA-I, apolipoprotein A-I; ApoB, apolipoprotein B; F, female; HDL-C, low-density lipoprotein cholesterol; HDL-P, high-density lipoprotein particle concentration; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; LDL-P, low-density lipoprotein particle concentration; Lp(a), lipoprotein(a); Lp-$PLA_2$, lipoprotein-associated phospholipase A2; SD, standard deviation; TCHOL, total cholesterol; TG, triglycerides.

*Indicates significant differences in the first and fifth quintiles compared with the third ($P < .0001$).

†The linear trends are characterized by Spearman's rho; significance is indicated by $P < .0001$.

**Table 2** Mean biomarker values (±SD) by discordance risk category (ApoB ≥ 69 mg/dL and/or LDL-P ≥ 1073 nmol/L), N = 412,013

| Patient characteristics | ApoB/LDL-P discordance risk categories | | | | ANOVA |
|---|---|---|---|---|---|
| | Both low n = 61,033 (15%) | High ApoB n = 21,415 (5%) | High LDL-P n = 25,023 (6%) | Both high n = 304,542 (74%) | (*P* value) |
| Demographics | | | | | |
| Gender (% F) | 48.2 | 58.0* | 44.9* | 50.7* | — |
| Age | 60.3 (16.3) | 58.1 (15.8)* | 60.6 (14)* | 56.2 (14)* | <.0001 |
| Lipids | | | | | |
| TCHOL (mg/dL) | 137 (25) | 174 (32)* | 138 (20)* | 197 (39)* | <.0001 |
| LDL-C (mg/dL) | 59.3 (13.1) | 82.7 (15.1)* | 67.8 (10.4)* | 113 (30)* | <.0001 |
| HDL-C (mg/dL) | 54.7 (17.7) | 63.0 (20.0)* | 46.9 (12.9)* | 52.1 (14.1)* | <.0001 |
| TG (mg/dL) | 100 (63) | 135 (195)* | 108 (47)* | 152 (98)* | <.0001 |
| Lipoproteins/apolipoproteins | | | | | |
| ApoB (mg/dL) | 56.9 (8.4) | 77.6 (8.4)* | 63.9 (4.8)* | 101 (23)* | <.0001 |
| LDL-P (nmol/L) | 818 (177) | 951 (110)* | 1220 (128)* | 1732 (467)* | <.0001 |
| LDL size (nm) | 20.9 (0.6) | 21.1 (0.6)* | 20.6 (0.5)* | 20.7 (0.6)* | <.0001 |
| ApoA-I (mg/dL) | 150 (33) | 162 (35)* | 139 (28)* | 146 (29)* | <.0001 |
| HDL-P (μmol/L) | 35.1 (7.1) | 36.7 (7.3)* | 34.2 (7.12)* | 34.3 (7.2)* | <.0001 |
| HDL size (nm) | 9.12 (0.56) | 9.27 (0.56)* | 8.73 (0.40)* | 8.7 (0.43)* | <.0001 |
| Lp(a) mass (mg/dL) | 28.3 (33.4) | 35.9 (41.8)* | 29.9 (35.7)* | 35.2 (41.1)* | <.0001 |
| Inflammation/metabolic | | | | | |
| hs-CRP (mg/L) | 3.2 (7.8) | 3.4 (8.1)* | 4.0 (9.1)* | 4.4 (8.5)* | <.0001 |
| Lp-$PLA_2$ (ng/mL) | 149 (44) | 161 (43.2)* | 137 (41.1)* | 148 (42.3)* | <.0001 |
| Insulin (μU/mL) | 14.2 (19.2) | 12.4 (17.5)* | 16.3 (20.2)* | 15.6 (19.1)* | <.0001 |

ANOVA, analysis of variance; ApoA-I, apolipoprotein A-I; ApoB, apolipoprotein B; F, female; HDL-C, low-density lipoprotein cholesterol; HDL-P, high-density lipoprotein particle concentration; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; LDL-P, low-density lipoprotein particle concentration; Lp(a), lipoprotein(a); Lp-$PLA_2$, lipoprotein-associated phospholipase A2; SD, standard deviation; TCHOL, total cholesterol; TG, triglycerides.

*Denotes significant differences compared with the "both low" group ($P < .0001$).

LDL-$P_{NMR}$ > apoB, bottom right) and fifth quintile (discordant apoB > LDL-$P_{NMR}$, top left).

Demographic and biomarker data are presented in Table 1 by ApoB/LDL-$P_{NMR}$ discordance score quintile (mean, standard deviation), with the first quintile representing the 20% most discordant (LDL-$P_{NMR}$ > apoB) and the fifth quintile representing the 20% most discordant (apoB > LDL-$P_{NMR}$). This method of presenting normalized discordance scores was chosen to investigate correlates of discordance independent of their absolute values. In comparing the first quintile (discordant, LDL-$P_{NMR}$ > apoB) with the third (most concordant) quintile, several trends are evident. Specifically, these patients tended to have lower lipid levels (total cholesterol, LDL-C, HDL-C, triglycerides [TG]), lower apoB and apoA-I values, smaller LDL-$P_{NMR}$ and HDL-P size, and lower Lp(a) mass (Table 1). Circulating levels of insulin and hs-CRP were higher in this group, though Lp-$PLA_2$ was lower. In contrast, the fifth quintile (discordant, apoB > LDL-$P_{NMR}$) was characterized by lower but larger LDL-$P_{NMR}$, higher HDL-C, larger and increased HDL-P, higher apoA-I, and lower hs-CRP and insulin, but higher Lp(a) mass and Lp-$PLA_2$. Analysis of these biomarkers as continuous variables revealed significant linear associations with ApoB/LDL-$P_{NMR}$ discordance score, as shown in Table 1. The strongest linear trends were observed for LDL size ($\rho = 0.32$, $P < .0001$), HDL-C ($\rho = 0.31$, $P < .0001$), total cholesterol ($\rho = 0.30$, $P < .0001$), and HDL size ($\rho = 0.29$, $P < .0001$).

The reason for this relatively high incidence of discordance between apoB assessment and NMR-determined LDL-P is not clear. Conceptually, because apoB is also a constituent of non-LDL atherogenic lipoproteins such as VLDL, IDL, and Lp(a), it could be hypothesized that discordance could result when these other apoB-containing particles constitute a higher than typical percentage of total apoB. In fact, we do report here an increase in Lp(a) mass in those discordant apoB > LDL-$P_{NMR}$ patients. However, because NMR does not typically differentiate Lp(a) or even IDL particles from LDL particles, this would require a much higher number of VLDL particles (and presumably of TG) than is ever seen, except in very rare cases resulting from genetic disorders. Our present data are not consistent with this explanation, because TG values were actually lower in the discordant group with higher than expected apoB compared with the third (most concordant) quintile, and the linear association between TG and discordance was close to zero.

The observations that the discordance scores are so normally distributed and linearly associated with several relevant biomarkers suggests an alternative hypothesis. Specifically, reduced LDL size, decreased HDL size and particle number, increased systemic inflammation, and increased insulin levels are all consistent with insulin

**Table 3** Patient characteristics by discordance risk category, N = 1411

| Patient characteristics | ApoB/LDL-P discordance risk categories | | | | ANOVA or Pearson (*P* value) |
|---|---|---|---|---|---|
| | Both low n = 383 (27%) | High ApoB n = 103 (7%) | High LDL-P n = 89 (6%) | Both high n = 836 (59%) | |
| Demographic | | | | | |
| Age (y) | 54.6 (14.2) | 55.5 (13.8) | 51.6 (15.1) | 51.9 (14.7)* | <.01 |
| Gender (% F) | 206 (54) | 65 (63) | 51 (57) | 515 (62)* | .08 |
| Caucasian‡ | 158 (68%) | 42 (67%) | 32 (52%) | 373 (68%) | .23 |
| Clinical | | | | | |
| BMI (kg/m$^2$) | 30.5 (6.6) | 31.6 (6.2) | 29.7 (7.1) | 30.6 (7.0) | .27 |
| Systolic BP (mmHg) | 122 (16) | 121 (15) | 121 (16) | 123 (17) | .37 |
| Diastolic BP (mmHg) | 74.8 (10.0) | 75.4 (10.6) | 76.3 (10.1) | 76.8 (10.6)* | <.05 |
| Currently smoking | 28 (7.3%) | 5 (4.9%) | 7 (7.9%) | 56 (6.7%) | .73 |
| LDL-C < 100 mg/dL | 382 (99%) | 99 (96%)* | 88 (99%) | 367 (44%)* | <.0001 |
| Lp(a) mass > 30 mg/dL | 137 (36%) | 45 (44%) | 35(40%) | 370 (44%)* | <.0001 |
| Medical history | | | | | |
| T2DM | 140 (37%) | 27 (26%) | 39 (44%)† | 211 (25%)* | <.0001 |
| Hypertension | 211 (55%) | 38 (37%)* | 51 (57%)† | 375 (45%)* | <.0001 |
| CAD | 108 (28%) | 18 (17%) | 16 (18%) | 106 (13%)* | <.0001 |
| Current medications | | | | | |
| Any antidiabetic | 222 (58%) | 39 (38%)* | 50 (56%)† | 341 (41%)* | <.0001 |
| Insulin | 59 (15%) | 11 (11%) | 14 (16%) | 105 (13%) | .41 |
| Metformin | 179 (47%) | 32 (31%)* | 35 (39%) | 252 (30%)* | <.0001 |
| Thiazolidinediones | 41 (11%) | 7 (7%) | 3 (3%) | 29 (3%)* | <.0001 |
| Secretagogues | 13 (3%) | 4 (4%) | 2 (2%) | 26 (3%) | .92 |
| Incretin mimetics | 92 (24%) | 17 (17%) | 23 (26%) | 101 (12%)* | <.0001 |
| Any lipid-lowering | 318 (83%) | 70 (68%)* | 78 (88%)† | 582 (70%)* | <.0001 |
| Statins | 284 (74%) | 53 (51%)* | 70 (79%)† | 399 (48%)* | <.0001 |
| Fibrates | 20 (5%) | 5 (5%) | 8 (9%) | 36 (4%) | .27 |
| Niacin | 87 (23%) | 20 (19%) | 15 (17%) | 93 (11%)* | <.0001 |
| Ezetimibe (Zetia) | 63 (16%) | 10 (10%) | 15 (17%) | 82 (10%)* | <.01 |
| Bile acid sequestrant | 19 (5%) | 3 (3%) | 8 (9%) | 62 (7%) | .13 |
| Any antihypertensive | 220 (57%) | 39 (38%)* | 55 (62%)† | 400 (48%)* | <.0001 |
| Any anti-inflammatory | 208 (54%) | 45 (44%) | 47 (53%) | 357 (43%)* | <.01 |

ANOVA, analysis of variance; ApoB, apolipoprotein B; F, female; BP, blood pressure; BMI, body mass index; CAD, coronary artery disease; LDL-C, low-density lipoprotein cholesterol; LDL-P, low-density lipoprotein particle concentration; Lp(a), lipoprotein(a); T2DM, type 2 diabetes mellitus.

Continuous and categorical variables are reported as "mean (standard deviation)" and "number of patients (% of discordance subgroup)," respectively.

*Denotes significant differences compared with the "both low" group ($P < .01$).

†Denotes significant differences between the two discordant groups ($P < .01$).

‡n = 911.

resistance or the metabolic syndrome. The effects of insulin resistance on lipoprotein profiles have been well characterized,[23,37] associated with production of smaller, denser, cholesterol-depleted—and potentially more atherogenic—LDL particles. It is possible, and to our knowledge not yet directly investigated, that the efficiency of current immunoassays for apoB may vary with respect to particle size or shape because of conformational changes in the binding epitope of apoB as the particle shrinks or distorts. It is also possible that an inflammatory milieu and/or metabolic disease can lead to oxidative, thermotropic, or glycative epitope changes, resulting in a false-negative apoB measurement.[38,39] Such a mechanism could explain why apoB appears to underestimate LDL particle number under conditions of insulin resistance.

## Clinical relevance of apoB/LDL-P discordance

To evaluate the potential clinical significance of this discordance (eg, determine how often it may affect a treatment decision), the same cohort was recategorized according to whether each patient had values above the 20th percentile of apoB (69 mg/dL) and/or LDL-$P_{NMR}$ (1073 nmol/L). As shown in Table 2, 15% of patients had "low-risk" levels of both markers, whereas 74% had "high-risk" levels of both. Importantly, 6% of these patients had risk identified by LDL-$P_{NMR}$ even though their apoB was considered normal, and 5% were identified to be at risk on the basis of apoB but had normal LDL-$P_{NMR}$ values. This relatively high incidence of discordance is not dependent on the particular cutpoint used to define risk; several other cutpoints were evaluated (eg, 50th

**Table 4** Mean biomarker values (±SD) by discordance risk category, N = 1411

| Biomarker | ApoB/LDL-P discordance risk category | | | | ANOVA (*P* value) |
|---|---|---|---|---|---|
| | Both low n = 383 (27%) | High ApoB n = 103 (7%) | High LDL-P n = 89 (6%) | Both high n = 836 (59%) | |
| Lipids | | | | | |
| TCHOL (mg/dL) | 129 (26) | 168 (24)* | 135 (21)† | 196 (43)* | <.0001 |
| LDL-C (mg/dL) | 55.2 (14.5) | 78.3 (14.0)* | 65.1 (11.6)*,† | 111 (33)* | <.0001 |
| HDL-C (mg/dL) | 54.8 (18.2) | 63.9 (19.8)* | 47.6 (13.0)*,† | 54.2 (15.5) | <.0001 |
| TG (mg/dL) | 88.5 (59.5) | 124 (125)* | 94.0 (43.7) | 137 (107)* | <.0001 |
| Lipoproteins | | | | | |
| ApoB (mg/dL) | 54.6 (9.8) | 75.9 (6.7)* | 62.8 (4.6)*,† | 99.3 (23.9)* | <.0001 |
| LDL-P (nmol/L) | 764 (197) | 903 (166)* | 1222 (115)*,† | 1684 (480)* | <.0001 |
| LDL size (nm) | 20.8 (0.6) | 21.0 (0.6)* | 20.5 (0.6)*,† | 20.7 (0.6) | <.0001 |
| ApoA-I (mg/dL) | 148 (31) | 157 (31)* | 142 (28)† | 148 (31) | <.01 |
| HDL-P (μmol/L) | 34.6 (6.5) | 35.2 (6.4) | 34.5 (6.7) | 34.1 (7.2) | .45 |
| HDL size (nm) | 9.16 (0.57) | 9.33 (0.62)* | 8.76 (0.44)*,† | 8.80 (0.47)* | <.0001 |
| Lp(a) mass (mg/dL) | 33.1 (36.3) | 41.2 (45.0) | 36 (40.1) | 42.3 (45.4) | .10 |
| Inflammation | | | | | |
| hs-CRP (mg/L) | 2.2 (3.3) | 4.0 (11.0) | 4.2 (6.8)* | 3.6 (6.6)* | <.0001 |
| Lp-$PLA_2$ (ng/mL) | 133 (39) | 158 (40)* | 127 (39)† | 144 (41)* | <.0001 |
| Metabolic | | | | | |
| Glucose (mg/dL) | 99.8 (34.4) | 97.3 (27.4) | 102 (29) | 102 (39) | .64 |
| HbA1c (%) | 5.8 (1.1) | 5.9 (1.2) | 6.0 (1.2) | 5.9 (1.4) | .47 |
| Insulin‡ (μU/mL) | 12.4 (9.2) | 12.2 (18.7) | 15.5 (17.2)*,† | 13.3 (13.3) | <.05 |
| C-peptide (ng/mL) | 2.97 (1.52) | 2.63 (1.35) | 3.4 (1.72)† | 3.01 (1.69) | <.05 |
| Proinsulin (pmol/L) | 16.1 (23.8) | 15.7 (20.0) | 21.3 (22.6)*,† | 18.3 (23.3) | <.01 |
| FFA (mmol/L) | 0.50 (0.24) | 0.57 (0.27)* | 0.52 (0.18) | 0.57 (0.24)* | <.0001 |
| Leptin (ng/mL) | 34.4 (35.5) | 32.2 (31.0) | 38.8 (38.9) | 34.7 (32.3) | .29 |
| Adiponectin (μg/mL) | 17.7 (19.9) | 16.6 (11.9) | 12.4 (9.69)*,† | 13.2 (10.1)* | <.0001 |
| Leptin:adiponectin | 3.36 (4.11) | 2.94 (3.50) | 4.96 (7.20)*,† | 3.83 (4.24)* | <.001 |
| HOMA-IR‡ | 2.97 (2.65) | 3.04 (6.22) | 3.98 (5.66)† | 3.41 (4.83) | <.05 |

ANOVA, analysis of variance; ApoA-I, apolipoprotein A-I; ApoB, apolipoprotein B; FFA, free fatty acids; LDL-P, low-density lipoprotein particle concentration; HDL-C, low-density lipoprotein cholesterol; HDL-P, high-density lipoprotein particle concentration; HOMA-IR, homeostatic model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; LDL-P, low-density lipoprotein particle concentration; Lp-$PLA_2$, lipoprotein-associated phospholipase A2; TCHOL, total cholesterol; TG, triglycerides.

*Denotes significant differences compared to the "both low" group ($P < .05$).

†Denotes significant differences between the 2 discordant groups ($P < .05$).

‡n = 1222.

percentiles) and similar proportions of discordant patients were identified (data not shown). It is clear from Table 2 that both discordant groups are associated with significant risk factors compared with the "both low" group. Particularly, the discordant (LDL-$P_{NMR}$ > apoB) group has smaller and more numerous LDL particles, higher inflammation, and hyperinsulinemia, whereas the discordant (apoB > LDL-$P_{NMR}$) group has elevated levels of Lp(a) mass.

Although intriguing, conclusions drawn from the observations described here are limited by a lack of patient clinical information, such as medical history and current treatment status. We therefore examined a separate cohort of 1411 well-characterized, aggressively treated patients at risk for cardiometabolic disease, with a high prevalence of insulin resistance. Discordance scores were calculated as described previously and were confirmed to have a distribution almost identical to that presented in Figure 2 (see Supplemental Fig. 1). Patient characteristics of this cohort are presented in Table 3, separated by discordance risk category using the same cutpoints of apoB ≥ 69 mg/dL and LDL-$P_{NMR}$ ≥ 1073 nmol/L. The mean age was 53 ± 15 years, 41% were male, 66% Caucasian, and mean BMI was 30.6 ± 6.8 kg/$m^2$. A high proportion of the sample had prior history of hypertension (48%), diabetes (30%), and coronary artery disease (18%), and was currently taking lipid-lowering (74%), antihypertensive (51%), and antidiabetic (46%) agents. A total of 27% of these patients currently had "low-risk" levels of LDL-P and apoB, whereas 59% had "high-risk" levels of both measures. Importantly, 6% of these patients had risk identified by LDL-$P_{NMR}$ even though their apoB was considered normal—an identical proportion to that identified in the larger cohort described previously. A similar number of patients (7%) were identified to be at risk on the basis of apoB but had normal LDL-$P_{NMR}$ values.

Laboratory biomarker values for these patients, also separated by discordance risk category, are shown in Table 4. Patterns of risk factors were similar to those observed in the larger cohort. Specifically, the discordant (LDL-$P_{NMR}$ > apoB) group had more numerous and smaller LDL particles and evidence of insulin resistance, compared with controls. It is worth noting that there was no evidence in this group of differences in glycemic control per se, nor of several other traditional markers of diabetes risk such as BMI, triglycerides, or even free fatty acids. However, significant differences noted for insulin, HOMA-IR, adiponectin, leptin:adiponectin ratio, and even proinsulin provide evidence of underlying insulin resistance that corresponds to the insulin-resistant lipoprotein profile. When compared with the discordant (apoB > LDL-$P_{NMR}$) patients, this group also shows significantly higher rates of type 2 diabetes mellitus incidence and antidiabetic treatment.

These observations illustrate the potential importance of apoB/LDL-$P_{NMR}$ discordance in a real-world setting where at-risk patients are being actively treated. It is very likely that medication use affects the degree of discordance observed here. For example, 79% of the LDL-$P_{NMR}$ > apoB group were taking statins, in contrast to 51% of the apoB > LDL-$P_{NMR}$ group. It has been shown previously that statins are better at lowering LDL-C than they are at lowering measures of LDL particles or apoB.[26] Because cholesterol-depleted particles tend to be smaller, the observation in the present study that discordance is linearly related to particle size suggests that statin use may be a significant factor in discordance between LDL-$P_{NMR}$ and apoB.

Regardless, 88% of the LDL-$P_{NMR}$ > apoB group were currently on lipid-lowering medication, and 99% had achieved LDL-C levels < 100 mg/dL; although measuring LDL-$P_{NMR}$ in these individuals may have revealed high numbers of atherogenic particles, an apoB test alone would not have identified their presumably significant residual risk. On the other side, the 7% of patients identified on the basis of apoB values when their LDL-$P_{NMR}$ was < 1073 nmol/L were more likely to have elevated plasma levels of Lp(a) and Lp-$PLA_2$. Interestingly, Lp-$PLA_2$ has been reported to show a stronger association for Lp(a) than for LDL particles.[40] The majority of these patients were also currently on lipid-lowering medication (68%) and 96% of them had LDL-C levels < 100 mg/dL, suggesting residual lipoprotein-related risk not identified by LDL-$P_{NMR}$ alone.

## Conclusion

The present data suggest that discordance between apoB and LDL-$P_{NMR}$ is more widespread in routine clinical practice than is currently recognized, and similar to (perhaps even slightly higher than) that reported in the literature review by Cole et al.[35] Discordance (LDL-$P_{NMR}$ > apoB) was associated with insulin resistance, smaller LDL particle size, increased systemic inflammation, and lower circulating levels of "traditional" lipids, whereas discordance (apoB > LDL-$P_{NMR}$) was associated with larger LDL particle size and elevated levels of Lp(a) and Lp-$PLA_2$. In both the large clinical dataset and the smaller at-risk cohort, similar proportions of patients were identified as at-risk by LDL-$P_{NMR}$ when their apoB levels were < 69 mg/dL (5%-6%) and by apoB values when their LDL-$P_{NMR}$ was <1073 nmol/L (6%-7%). Until this discordance can be correlated with clinical outcomes, it is not possible to declare with certainty which particle biomarker (if either) is the false positive or false negative. Our analysis from a very large database suggests that both can be helpful in the comprehensive assessment of cardiovascular risk related to increased atherogenic particle burden, particularly when insulin resistance or Lp(a) abnormalities are present.

## Acknowledgments

This work was supported by Health Diagnostic Laboratory, Inc.

## Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jacl.2014.11.005.

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